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STUDIES OF ALTERED RESPONSE TO INFECTION INDUCED BY SEVERE INJURY

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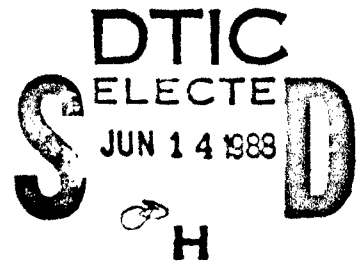
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## Introduction

The purpose of this research is: (1) to define the role of monocyte and T cells and their products - different cytokines in mediating immunosuppression in post trauma patients; (2) to develop and improve assays for assessment of MØ functions and to detect different monokine productions; (3) to study the relationship between immunosuppression and post trauma alterations in MØ subsets, and (4), to investigate prophylactic modalities for reversing immunosuppression.

During this contract year, we have made significant advances in pursuing these goals. First, we have demonstrated that trauma induced suppressor T cells (T<sub>s</sub>) can depress MØ functions, and this T<sub>s</sub> mediates immunosuppression by inducing a shift in the MØ subsets toward a subset with augmented prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) producing capacity, as well as an increased tumor necrosis factor (TNF) activity. Second, we made tremendous progress in goal two; we have initiated the bioassay for measurement of MØ TNF production. TNF is known to be involved in cachexia and its elevation is associated with endotoxin shock. The new established method allows us to detect an increased TNF level in immunosuppressed patients. We have also successfully switched our PGE<sub>2</sub> assay from an RIA to a more sensitive ELISA. This ELISA is more reproducible from assay to assay. We are using a densitometer which reads the ELISA plates and transfers the data directly into our Compaq computer. As mentioned above, we have shown that elevation of MØ PGE<sub>2</sub> and TNF production appears to correlate with a shift towards a MØ subset that can be identified by its expression of the high affinity receptor for human IgG1, human IgG3, and mouse IgG2a. This shift in MØ subpopulations corresponds to an increase in these patients' MØ production of PGE<sub>2</sub> and TNF consistent to a depression of their antigen presenting function and plasminogen activator activity. This is the reason that we have pursued goal three. If we can associate a change in MØ function with a change in MØ phenotype, the increased appearance of this particular subset could then quickly detect the onset of immunoincompetence in trauma patients. Such a quick and easy detection of immunoincompetence would have application to identification and segregation of combat victims requiring more intensive care. Our data have demonstrated that there is an increase in the number of FcRI<sup>+</sup> MØ subset and that this increase correlates both to an elevation of PGE<sub>2</sub> and TNF production and poor clinical outcome. Finally, in pursuing the goal of testing new prophylactic therapies, we have received animal experimentation approval for the thermally injured guinea pig model and have performed several experiments using this model. We intend to examine the effect of ibuprofen on the development of increased MØ TNF, PGE<sub>2</sub> production and decreased MØ PA activity. We have examined the effect of the MØ stimulator, muramyl dipeptide (MDP), on patient's MØ function in vitro. The MDP stimulated MØ still exhibited depressed PA activity and elevated PGE<sub>2</sub> and TNF production. This MDP mediated

result is similar to the suppressive effect we see mediated by early patient Ts. The MDP model may be relevant to bacterial product mediated suppression and Ts mediated suppression in these patients. When indomethacin was added to the MØ cultures simultaneously with MDP, the augmented PGE<sub>2</sub> production of the FcRI<sup>+</sup> MØ population was abolished. Indomethacin also partially abrogated the MDP mediated suppression of FcRI<sup>+</sup> MØ PA activity. We will be examining the possibility that some other prostacyclin besides PGE<sub>2</sub> (such as leukotriene B<sub>4</sub>), is mediating the suppression.

In summary, this has been a highly productive year. We have established several new methods to monitor immunosuppression developed in severely injured patients and have demonstrated that immunosuppressed patients have elevated MØ PGE<sub>2</sub> and MØ TNF production in conjunction to depressed MØ PA activity and poor clinical outcomes.

## Methods

The Plasminogen Activator (PA) assay is performed as previously described: (1) The procoagulant assay and the lysozyme assay are also performed as previously described (1-2). The assay for suppression of MØ PA activity is performed by isolating the MØ as described (1), then recombining either  $2 \times 10^6$  normal or  $2 \times 10^6$  patient MØ with equal numbers ( $2 \times 10^6$ ) of the patients E-rosetted T cells. The mixed populations are co-cultured for 48 hours in Iscove's supplemented with 8% FCS and media supplements as described (1). In most assays, the normal and patient MØ are collected at day 1-10 post injury and cultured in supplemented Iscove's for 3-4 days. The patient T cells collected at 3-5 (early) or 13-22 (late) days post injury are co-cultured with the normal or patient MØ collected 3-4 days earlier.

MØ PGE<sub>2</sub> is measured with a newly developed enzyme-linked immunosorbant assay (ELISA) for PGE<sub>2</sub> detection in our laboratory this year. Microtiter 96 well plates (Nunc, VWR) were coated with 200 µl/well goat anti-rabbit IgG in  $5 \times 10^{-1}$  M potassium phosphate buffer pH 7.4 (Jackson Immuno Research Labs. Cat# 111-005-045) for 18 hours at room temperature. After extensive washing plates were saturated with enzyme immunoassay (EIA) buffer at 4°C for 18 hours. In the competitive binding assay PGE<sub>2</sub> standard (Sigma) or sample were added first followed by the acetylcholinesterase - PGE<sub>2</sub> (Enzyme tracer) conjugate (ACE<sup>TM</sup>) (Commissariat à l'Energie Atomique, Institut de Recherche Fondamentale, Saclay, France), and rabbit anti-PGE<sub>2</sub> antibody (Sigma, P3038) for 18 hours at room temperature. Bound acetylcholinesterase activity was measured by adding enzyme substrate (Ellman's reagent) (Sigma) to the wells. Optical density of each well was detected at 410 nm on a Dynatech MR600 plate reader. Calibration curve was created from the dilution of PGE<sub>2</sub> standard and the appropriate % of fraction bound ( $B/B_0$  %) was calculated by the following formula using our compaq computer which is connected with the densitometer.  $B/B_0$  % =  $(ST \text{ or } SMP - NSB) / (B_0 - NSB) \times 100$ , where NSB represents the nonspecific binding,  $B_0$  is the mean absorbance of wells which have reached the maximum density, ST is the mean of each standard and SMP is the mean of each sample.

Determination of MØ tumor necrosis factor (TNF) production is performed by isolating the MØ as described (#1), then MØ are pre-stimulated with 10-100 µg/10<sup>6</sup> cells of human recombinant interferon-gamma (IFN<sub>γ</sub>) (obtained from Collaborative Res. Inc.) followed by a second 20 µg/ml MDP stimulation, 5-6 hours later. In the patients' assays, nor-MDP (a generous gift from Ciba-Geigy, Basel, Switzerland), an MDP derivative was used, which has greater activity inducing cytotoxicity of MØ than MDP itself. Cells are cultured in Iscove's media supplemented with 15% FBS, antibiotics, fungizone and L-glutamine at 37°C. After 20 or 40 hours, culture cell-free supernatants are collected and kept at -80°C until further use. TNF and PGE<sub>2</sub> levels are determined in the supernates. Cells are deattached by EDTA treatment and scraping and kept frozen. After repeating freeze-thawing followed

by sonication, intracellular TNF and procoagulant activity (PCA) are determined.

Tumor necrosis factor was assayed using serum-free *in vitro* bioassay described by Kramer and Carver (#2) with slight modifications. Murine L-M connective tissue cells, a derivative of L929, were obtained from ATCC (CCL1.2) and grown in medium 199 supplemented with 0.5% Bacto-Peptone (M199/0.5% Peptone) (Difcon-laboratories, Detroit, MI), 1.35mg/ml sodium bicarbonate and antibiotics. Cell monolayers were detached with 0.25% trypsin treatment (GIBCO) extensively washed and subcultured every 3-5 days using  $10^5$  cell/ml density. A 96 well flat bottom microtiter plate (Costar) was seeded with  $3 \times 10^4$  cells/well in a 100 $\mu$ l volume of M199/0.5% Peptone, leaving well A1 cell free. Following a 22-24 hour incubation at 37°C in a specialty gas mixture (10% CO<sub>2</sub>, 7% O<sub>2</sub>, 83% N<sub>2</sub>), medium was removed and replaced with fresh M199/0.5% Peptone. 100 $\mu$ l of TNF sample, standard or control was added to wells 1-12 of row A in duplicate. Serial two-fold dilutions were prepared from row A through row H, and 100 $\mu$ l volume from row H was discarded. Finally, 100 $\mu$ l medium containing 2 $\mu$ /ml Actinomycin-D (Sigma, A-1410) was added to all wells. Plates were incubated 20-22 hours at 39°C. Plates were stained with 0.5% crystal violet (0.5g crystal violet in 20% methanol) (Sigma, C-6158). Excess stain was removed with repeated washing in tap water. Absorbance was determined at 570nm using a Dynatech MR600 plate reader.

Titers were expressed as the reciprocal of the dilution resulting in 50% cytotoxicity determined from the mean absorbance of 11 control wells in each plate. The endpoint corresponding to the 50% cytotoxicity absorbance value was used to calculate sample activity using the following formula:

$$\text{Sample Act (pg/ml)} = \text{Std Act (pg/ml)} \times \frac{\text{Sample EP} - \text{STD EP}}{\text{STD EP}}$$

MØ subsets are separated based on the density of high affinity Fc receptors for IgG2a, by rosetting MØ with anti-Rh coated human erythrocytes (Zembaia et al) MØ subsets are stimulated the same was as that was described for the whole MØ population. Subset experiments are performed on trauma and burn patients to delineate the differential stimulation requirements and monokine production capacity of MØ subsets. We used cyclo-oxygenase inhibitor indomethacin at a concentration of  $1 \times 10^{-6}$  M to block the MDP induced PGE<sub>2</sub> production of normal and patient MØ and MØ subsets.

<sup>1</sup> Miller, et al, 1982, J. Immun., 128:2194

<sup>2</sup> Kramer and Carver, 1986, J. Immun., 93:201



## Results and Discussion

In the period covered by the second year of contract No. DAMD17-86-C-6091, several goals have been attained. Twenty-six patients have been nominated this year, including 14 trauma and 12 burn patients.

Of these patients four were studied in the first four months, eleven the next five months and eleven the last four months of the year. Of these patients two burn patients and four trauma patients have succumbed to fatal sepsis.

As illustrated in Table 1, we found reduced MØ functions in the burn and trauma patients who experienced immunodepression. As we have previously shown, there is a correlation between MØ PA depression and depression of phytohemagglutinin (PHA) induced T cell proliferation concomitant with elevation of MØ prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) levels and poor prognosis. As can be seen, there is a change in MØ PGE<sub>2</sub> and depression of MØ PA in some trauma as well as burn patients who experience no septic complications.

Experiments reported last year indicated an increased MØ PGE<sub>2</sub> production as a result of exposure of patient or normal MØ to T suppressor cells (T<sub>s</sub>) collected from immunosuppressed patients as detailed in the methods. At that time we were hypothesizing that immunosuppression experienced by trauma and burn patients could be related primarily to elevated MØ PGE<sub>2</sub> levels. These increased MØ PGE<sub>2</sub> resulted from a shift in FcRI positive and negative MØ subsets towards the PGE<sub>2</sub> producer, Fc<sup>+</sup> subset. The other possible reason for the increased MØ PGE<sub>2</sub> in these immunoincompetent patients is the appearance of a non-specific T<sub>s</sub> which increased MØ PGE<sub>2</sub> production. We have confirmed that the late appearing non-specific T<sub>s</sub> which is CD8<sup>+</sup> does increase MØ PGE<sub>2</sub> production (Table II). However, we have determined that a CD4<sup>+</sup> T<sub>s</sub>, arising early in the post-injury period, suppress MØ function but does not increase MØ PGE<sub>2</sub> production (Table II). This early T<sub>s</sub> appears to have a Fc<sup>-</sup> MØ as its target.

We have similar data showing the MØ induction by muramyl dipeptide (MDP) in patients and normals can suppress MØ functions by both increasing PGE<sub>2</sub> and by a non-PGE<sub>2</sub> mediated mechanism (Table 3). We have examined the stimulation capacity of MDP on patient MØ and MØ subsets to detect early post-trauma change in MØ responsiveness to bacterial stimuli. MDP is a potent PGE<sub>2</sub> secretagogue and we, and others have found that it also depresses MØ PA activity. In our last annual report we have already suggested the importance of a shift in MØ subsets in the post-injury immunosuppression. We examined the

subset ratios of patients MØ populations by separating out the MØ subset rosetting with the high affinity receptor for IgG2a/FcRI<sup>+</sup>). This FcRI<sup>+</sup> subset has been previously suggested to contain the inhibitory MØ population. As we reported in our recently published paper (SIS, March), immunosuppressed patients had a dramatic increase in the proportion of their FcRI<sup>+</sup> MØ subset which coincided with an increase in their PGE<sub>2</sub> activity. In our recent experiments done in the last quarter of this year we used indomethacin *in vitro* to block MDP induced MØ PGE<sub>2</sub> production by patient MØ. As can be seen in table 3, a cyclo-oxygenase inhibitor, indomethacin reverses MDP inhibition of MØ PA responses in both FcRI positive and negative subsets, suggesting that FcRI<sup>+</sup> MØ PA depression of MDP is possibly mediated by another cyclo-oxygenase product (eg. LTE<sub>4</sub>) and not by PGE<sub>2</sub>, or by some other factor(s). However in the FcRI<sup>+</sup> MØ population, MDP induced PA depression was reversed by indomethacin to the normal level. These preliminary data coming from experiments done on two patients need to be confirmed in the next quarter. Since ibuprofen therapy is now being used in clinical trials in trauma and burn patients, we need to understand the possible advantages and disadvantages in its action.

We made a progress this year in testing MØ functions of post-injury patients developing a bioassay for determination of tumor necrosis factor (TNF). TNF-alpha, also called cachectin, is a monokine produced by stimulated monocytes, having a major role in the metabolic development in cachexia and septic shock. Therefore we examined the TNF production of patients and normal monocytes and MØ subsets after different stimuli. As shown in Table 4, stimulation of patients or normal MØ with MDP or IFN $\gamma$  alone could not induce TNF production. However, combination of stimuli, IFN $\gamma$  plus MDP induced both patients and normal MØ TNF production. In developing the TNF assay for patient populations, we found that maximal stimulation of TNF with 20 $\mu$ g/ml of MDP and 100U/ml of IFN $\gamma$  often obscured differences in response between patients and normals. In contrast, suboptimal stimulation with IFN $\gamma$  (10U/ml) + MDP resulted in a large response from immunosuppressed patient MØ while only a minimal response was stimulated in the normal population. Using this protocol we have also demonstrated that the FcRI<sup>+</sup> MØ population in both controls and immunocompromised patients produces the majority of TNF in response to stimulation by bacterial cell wall products and IFN $\gamma$  (Table 5). This is a significant new finding and important because of the shift toward FcRI<sup>+</sup> MØ population seen in immunocompromised patients. The post trauma *in vivo* environment contains both bacterial cell wall products and activated by lymphokines.

These stimuli would induce increased TNF production in patients with a shifted MØ FcRI<sup>+</sup> subset ratio and could cause cachexia and metabolic derangement. Although Dr. Faist (personal communications) has indicated that elevated TNF levels occur in his patients, no published data demonstrating elevated MØ TNF in immunocompromised trauma patients have appeared in the literature. Our data (Table 4) clearly shows an increase in immunosuppressed patients' TNF levels. These patients also have increased leukocyte pyrogen activity which may be the result of their elevated MØ TNF production. We interpret this

data as implying that the increased TNF production by the immunosuppressed patients' MØ population may be accounted for by the presence of greater numbers of Fc<sup>+</sup> MØ.

Preliminary experiments are also underway examining the effect of blocking cyclo-oxygenase products in these patients. Since MØ PGE<sub>2</sub> production is a major contributor to post trauma immunosuppression, it is reasonable to consider blocking the synthesis of this cyclo-oxygenase product by administering ibuprofen to patients. In vitro blocking of MØ PGE<sub>2</sub> production with indomethacin resulted in an unexpected effect on MØ TNF production. We are examining both secreted and intracellular TNF and finding that normal MØ respond by secreting most of their TNF (TABLE 5). Intracellular TNF also rises in the normal MØ after stimulation but not to levels above those secreted. In contrast, in studies on two immunosuppressed patients' MØ we found massively increased intracellular TNF levels as well as increased secreted TNF. Addition of indomethacin to these patients' MØ actually resulted in increasing their MØ TNF release. If these results are consistently found in further studies, it may present a problem for clinical administration of cyclo-oxygenase inhibitors to immunosuppressed trauma patients. Increased TNF release with consequent endotoxin shock might result from such ibuprofen therapy. With the aid of Dr. Thomas Takayama, we are moving forward with the thermally injured guinea pig model. We will test MØ TNF production after thermal injury in this model and examine the in vivo effect of administering ibuprofen to these animals. At a recent meeting it was reported that endotoxin shock was occurring in some patients receiving ibuprofen. This information makes testing both the patient MØ TNF response after ibuprofen and the guinea pig MØ response critical to evaluating clinical trials. The shift in MØ subsets we have identified have several implications. The patients exhibiting such a MØ subset shift will experience increased TNF levels, increased PGE<sub>2</sub> levels, depressed Antigen presenting function and depressed PA activity. The ability to identify these shifts in patients MØ populations should significantly simplify the monitoring of trauma patients immune profile and be directly applicable to identification of combat casualties at risk of infectious complications. In addition, development of a sensitive assay for patient MØ TNF levels will improve ability to detect the onset of endotoxin shock in trauma victims.

Table 1

CORRELATION OF MØ PA DEPRESSION, PGE<sub>2</sub> AUGMENTATION AND CLINICAL OUTCOME

<u>Pt.</u>	<u>Injury</u>	<u>ΔPHA%</u> <sup>a</sup>	<u>PA (01-13)</u> <sup>b</sup>	<u>ΔPGE<sub>2</sub> ng</u> <sup>c</sup>	<u>Outcome</u>
1. Do	burn	-42	53.7→39.7	4.2→48.5	No Compli- cations
2. Ho	burn	-28	39.0→11.2	3.5→54.6	Multiple Septic Episodes
3. Sh	burn	-37	41.7→31.3	12.5→20.2	No Compli- cations
4. Lo	burn	-43	31.3→28.1	3.9→23.5	No Compli- cations
5. To	burn	-28	32.0→17.2	6.9→42.0	Multiple Septic Episodes
6. Cr	burn	-80	54.8→17.0	9.5→15.9	No Compli- cations
7. Al	burn	-66(11)	30.2→9.1	ND. <sup>d</sup>	Fatal Sepsis
8. Cr	burn	-74(12)	33.9→16.8	13.2→32.0	Multiple Septic Episodes
9. Ho	burn	-75(8)	35.7→14.9	54.0(15.0)	Multiple Septic Episodes
10. Sm	burn	-90(7)	19.6→7.7	6.2→10.0	Fatal Sepsis
11. Al	trauma	-63	31.4→24.4	8.3→20.3	No Compli- cations
12. Er	trauma	-49	50.1→32.3	6.2→24.5	No Compli- cations
13. Ca	trauma	-75	53.5→23.2	10.5→44.3	Died
14. Sc	trauma	-14	26.3→20.7	11.0→36.0	No Compli- cations

15. Fo	trauma	-84	38.8→18.3	4.1→29.0	Died
16. Tu	trauma	-82	37.5→11.0	6.2→51.0	Infectious Episode
17. Bu	trauma	-76(8)	30.8→8.8	→46.00	Septic
18. Ch	trauma	-66 <sup>*</sup>	28.3(27.0)	N.D.	Fatal Sepsis
19. Je	trauma	-47(4)	16.8→10.3	34.0→37.00	Septic Compli- cation
20. Yo	trauma	-91(5)	37.1→10.6	3.6→16.00	Multiple Septic Episodes

a) Maximum change at 2-12 days post injury in mitogen induced proliferation of  $2 \times 10^5$  peripheral blood mononuclear cells to  $2 \mu\text{g}$  PHA.

b) Maximum change in MØ plasminogen activator (PA) activity from initial response to most depressed within the first 13 days post injury. PA activity measured in % plasmin specific fibrinolysis.

c) Maximum prostaglandin  $E_2$  production in first 13 post injury days measured as nanograms per  $10^6$  recovered MØ.

d) Not done.

Table 2

COMPARISON OF T<sub>H</sub> ACTIVITY OF EARLY AND LATE T CELLS FROM  
IMMUNOSUPPRESSED PATIENTS<sup>a</sup>

T Cell Source	PA(% fibrinolysis) <sup>b</sup>	PGE <sub>2</sub> pg/10 <sup>6</sup>	MØ(x10 <sup>-3</sup> ) <sup>c</sup>
MØ Source	Days Post Injury <sup>d</sup>	MØ	MØ+T <sub>H</sub>
Norm	22	43.3 → 25.7	6.2 → 17.1
Pt	22	27.8 → 20.1	13.1 → 19.9
Norm	21	40.9 → 17.7	8.4 → 24.3
Pt	21	26.0 → 20.6	20.6 → 28.0
Norm	16	34.5 → 16.5	11.3 → 17.4
Pt	13	28.7 → 13.4	14.6 → 30.5
Norm	5	56.2 → 38.4	1.7 → 1.7
Pt	5	29.8 → 22.2	2.6 → 3.9
Norm	3	50.0 → 29.4	3.6 → 3.5
Pt	3	36.4 → 26.3	4.0 → 4.9

- EA-rosetted, MØ depleted T cells collected from patients with depressed MØ PA activity (immunosuppressed) were co-cultured with equal numbers of autologous patients or allogeneic normal MØ for 48 hours. (see Methods)
- PA responses of MØ cultured alone or MØ co-cultured with patient T cells.
- PGE<sub>2</sub> responses of MØ cultured alone or MØ co-cultured with patient T cells.
- Day of T cell collection either late (>12 days) or early (>10 days). T cells recombined with MØ collected 2-3 days earlier.

Table 3

NON PGE<sub>2</sub> MEDIATED MDP INHIBITION OF MØ SUBSET PA RESPONSES

<u>MØ SOURCE</u> <sup>1</sup>	<u>PA (% fibrinolysis)</u>	<u>PGE<sub>2</sub> ng/10<sup>6</sup> MØ</u>
Total	34.4±1.6	32±12
Total + MDP <sup>2</sup>	25.0±2.1	72±23
FcR1 <sup>+</sup>	26.0±5.4	31±8
FcR1 <sup>+</sup> + MDP	14.6±3.1	76±24
FcR1 <sup>-</sup>	39.0±6.7	4.0±0.8
FcR1 <sup>-</sup> +MDP	29.2±7.4	5.5±1.9

Indomethacin reverses MDP inhibition of MØ PA responses in patient:

<u>MØ Source</u>	<u>PA (% fibrinolysis)</u>
FcR1 <sup>+</sup> + MDP	11.3
FcR1 <sup>+</sup> + MDP + Indo <sup>3</sup>	20.2
FcR1 <sup>-</sup> + MDP	10.6
FcR1 <sup>-</sup> + MDP + Indo	25.2

1. Unseparated MØ, or MØ rosetted with α Rh coated human erythrocytes = FcR1<sup>+</sup> MØ, non-rosetting = FcR1<sup>-</sup>
2. MØ stimulated with 20µg/ml MDP
3. MØ cultures contain 20µg/ml MDP and 10<sup>-6</sup> M Indomethacin

Table 4  
INCREASED TNF<sup>1</sup> PRODUCTION BY IMMUNOSUPPRESSED PATIENTS<sup>2</sup>

<u>MØ Source</u>	<u>Day Post-Injury</u>	<u>Stimulated with:</u>		
		<u>MDP<sup>3</sup></u>	<u>IFN<math>\gamma</math><sup>4</sup></u>	<u>IFN<math>\gamma</math> + MDP<sup>5</sup></u>
pty	27	0	0	3582
norm		0	0	459
ptH	15	0	0	1679
norm		0	0	0
pts	16	0	0	2015
norm		0	0	0

norm max. stim.<sup>6</sup>:

$\bar{x}$  2643+1395

1. Tumor necrosis factor measured as pg produced by 10<sup>6</sup> MØ.
2. Immunosuppressed patients defined as having depressed MØ PA and PHA responses.
3. MDP was used at 20 $\mu$ g/ml concentration (suboptimal level) to stimulate MØ for 20 hours.
4. 10 $\mu$ /10<sup>6</sup> MØ IFN $\gamma$  was the stimuli for MØ.
5. Combination of 10u 1FN $\gamma$ /10<sup>6</sup> MØ with 20 $\mu$ g/ml was used for stimulation of MØ TNF.
6. Maximal stimulatory dose: 100u of IFN $\gamma$ /10<sup>6</sup> MØ plus 20 $\mu$ g/ml MDP.



Table 5

INCREASED TNF PRODUCTION BY PT MØ/Fc<sup>+</sup> SUBSET

		Stimuli		
		None	5U IFN <sub>γ</sub> + 20μgMDP	100U IFN <sub>γ</sub> + 20μgMDP
NORMAL		398	993	1908
PT		1218	2170	6706
FC <sup>+</sup> NORMAL		104	1396	8708
PT		1638	4825	20268
FC <sup>-</sup> NORMAL		0	608	1029
PT		0	392	2779

Table 6

TNF PRODUCTION BY MØ SUBSETS AT 20 HOURS  
TNF pg/10<sup>6</sup> MØ

	<u>Secreted</u>	<u>Intracellular</u>	<u>Total</u>
MØ	0 <u>+0</u>	0 <u>+0</u>	0
MØ+IFN $\gamma$ +MDP <sup>1</sup>	2643 <u>+1395</u>	1992 <u>+1233</u>	4635
MØ+IFN $\gamma$ +I1-2 <sup>2</sup>	1399 <u>+810</u>	1729 <u>+1588</u>	3227
FCR1 <sup>+</sup>	243 <u>+387</u>	572 <u>+895</u>	815
FCR1 <sup>+</sup> +IFN $\gamma$ +MDP	6995 <u>+4023</u>	3218 <u>+997</u>	10213
FCR1 <sup>+</sup> +IFN $\gamma$ +I1-2	2854 <u>+1733</u>	2991 <u>+1608</u>	5844
FCR1 <sup>-</sup>	21 <u>+51</u>	225 <u>+558</u>	243
FCR1 <sup>-</sup> +IFN $\gamma$ +MDP	3960 <u>+3989</u>	1407 <u>+928</u>	5366
FCR1 <sup>-</sup> +IFN $\gamma$ +I1-2	1813 <u>+1533</u>	1961 <u>+1500</u>	3744

1. MØ were stimulated with 100U/10<sup>6</sup> cells of IFN $\gamma$  plus 20 $\mu$ g/ml MDP
2. MØ were stimulated with 100U/10<sup>6</sup> cells of IFN $\gamma$  plus 1000U/10<sup>6</sup> cells of I1-2

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